

Project No. _____

Book No. _____

TITLE _____

134

From Page No. _____

48 μ l of premix aliquoted to pre labeled appendants 4/1

1	1/200	1	} post heat kill
2		2	
3	1/500	1	
4		2	

5	1/500	1	} pre heat kill
6		2	
7	1/500	1	
8		2	

incubate @ 74°C in a heated water block - for 10 minutes
quench w/ 10 μ l of 5M EDTA - spot 30 μ l on GPC

Wash 1x 10% TCA 1x Pi 5'
3x 5% TCA
2x 5% H

dry + count w/ Econofluor

USER: 2 ID: 32P
SAMPLE REPEAT:
H#: 1 AQC: N QCF
CHANNEL 1-LL:
DATA CALC: CPM.
HALF LIFE (DAYS):

SAM	CPM1
1/200	8460.00
2	21680.00
1/500	8296.00
4	7486.00
1/200	16274.00
6	28614.00
1/500	8412.00
8	17912.00
9	2794.00
10 S.A.	91294.00

} post heat kill	11.9
	17.88
	29.7
	28.4
} pre heat kill	30.1
	29.54
	29.7

S.A. 57 cpm/pmol at

mf
6/20/95

To Page 1

Witnessed & Understood by me,

Date

Inv. nted by

Date

May Jones

6/20/95

R c rd d by

Elizabeth

06/19/95

'ag N .__

27. SDS PAGE - 3-5' exo minus the



5/10/14

12/6/20/55

1	2	3	4	5	6	7	8	9	10
1 _μ	3 _μ	5 _μ	5 _μ	10 _μ	20 _μ		1 M		
pre heat kill			post heat kill						

u heat - @ 20 $\mu\text{g}/\mu\text{l}$ - spun down sup loaded on gel
st heat @ .36 $\mu\text{g}/\mu\text{l}$

To Page No._____

ssed & Understood by m ,

May Long

Date _____

6/20/95

Invented by

Record d by

3.4 gm

Date

06/16/95

Project No. _____

Book No. _____

TITLE

FY1 - S. Sgm crack

From Page No. _____

Dur. pose

to screen FY1 - mutant Tne - one base
point mutation phenylalanine to Tyrosine 1 for
thermostable polymerase activity.

S. Sgams cells - resuspend in 10 mL of crack buffer - p. 132 ~ 15

Divide in two 7.5 mL samples in 15 mL conical
Sonicate w/ microtip @ max output - 5
6 x 20 sec bursts

Before - A₅₉₅ .750
After - A₅₉₅ .320 ~ 57% crack

Sonicate again - 3 x 20 sec bursts

Before A₅₉₅ .750 ~ 73% crack
After A₅₉₅ .200

Divide Save 400 mL - pre crack material
Aliquot remainder of crack into 2 mL eppendorf
incubate @ 87°C 11 minutes -
Spin in microfuge 20 minutes @ 14,000 ³

Decant and save supernatant → assay for
thermostable polymerase activity -

To 500 μ L of TAD premix add 1.1 μ L of ^{p32} dCTP

48 μ L of premix / rxn - 1.2 μ L of
diluted samples added incubate @
74°C in a heated water block -
for 10'. Quench rxn w/ 10 μ L of

1	1/200	1
2		2
3	1/400	1
4		2
5	1/200	1
6		2
7	1/400	1
8		2

5.0 M EDTA - spot 30 μ L of GFI C
Wash 1x w/ 10% TCA (1% Pi @ 5'
3x w/ 5% TCA @ 3'
1x w/ EDTA

dry + count -

To Page No.

Witness d & Understood by m ,

Date

Inv nted by

Date

Rec rded by

May Longo

6/20/95

E. Flynn

6/16/95

Page N. _____

USER: 2 ID: 32P
 SAMPLE REPEAT:
 H#: 1 ACC: N QCF
 CHANNEL 1-LL:
 DATA CALC: CPM
 HALF LIFE (DAYS)

Note: Background very high!

06/14

Debris note: Did not induce for very long -

SAM CPM1

✓ 1 5722.00
 2 7676.00
 3 2608.00
 4 4686.00
 5 10454.00
 6 19114.00
 7 5850.00
 8 11594.00
 9 2976.00
 10 90102.00
 11 88418.00

Post

5.8

5.4 U/ul

Heat Kill

5.0

loss of 70%?

Pre Heat Kill

15.9 U/ul

17.1 U/ul

17.0 U/ul

18.3

my 6/20/95

Appears to have (very high background) +
 lost activity after heat kill
 * maybe mostly lost D1 activity in pre heat kill?

Brad finds

Slope - .0555 0.0/ug?

ul

0.0.

19/ul

Heat Kill Tyl - 40

.474

* too ↑

.2 ug/ul

20

.333

.289 ug/ul

Pre Heat Kill Tyl - .

.259

.439

23.3 ug/ul

19.8

Post Heat Kill

.303

.136 ug/ul

3-5' zero mat

Run 12x SDS PAGE - see p 138

1 - 2 - 3 - 4

↓

To Page No. _____

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May Longo

6/20/95

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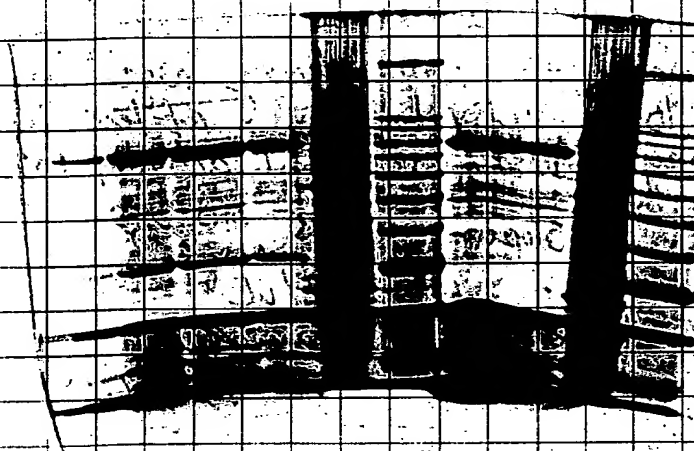
TITLE FYI + 3'-5' exo mutant SDS PAGE

From Page No. _____

06/11

12% SDS PAGE

1 2 3 4 5 6 7 8 9 10

1 6 μ g 4 μ g 2 μ g 60 μ g M 4 μ g 2 μ g 60 μ g MFYI
post heat
killFYI
pre heat
kill3'-5' exo M
post heat
killpre heat
kill 3'-5' exo-

100kd

50kd

DRT 6/20/95

GTF
C

pre heat kill - spun down sup loaded on gel

To Page 1

Witnessed & Understood by me,

Date

Invented by

Date

May Longo

6/20/95

Record d by

S. Flynn

06/16/95